

What Is Claimed Is:

1. A method of identifying an agonist or an antagonist of a co-regulator-dependent target molecule comprising providing a set of molecules that modify the stability of the target molecule and screening one or more molecules of said set for their ability to further modify the stability of the target molecule in the presence of one or more co-regulators; wherein a further modification of the stability of the target molecule in the presence of a molecule of said set and a co-regulator of said one or more co-regulators indicates whether the molecule is an agonist or an antagonist of the target molecule when in the presence of said co-regulator.

2. The method of claim 1, wherein providing the set of molecules that modify the stability of the target molecule comprises screening one or more of a multiplicity of different molecules for their ability to modify the stability of the target molecule.

3. The method of claim 2, wherein the screening of said one or molecules of said set of molecules for their ability to further modify the stability of the target molecule comprises:

(a) contacting said target molecule and one or more molecules of said set with one or more of said co-regulators in each of a multiplicity of containers;

(b) treating said target molecule in each of said multiplicity of containers to cause said target molecule to unfold;

(c) measuring in each of said containers a physical change associated with the unfolding of said target molecule;

(d) generating an unfolding curve for said target molecule for each of said containers;

(e) comparing each of said unfolding curves in step (d) to (1) each of the other unfolding curves and/or to (2) the unfolding curve for said target

molecule in the absence of (i) any of said molecules of said set and/or (ii) said co-regulators; and

(f) determining whether any of said molecules of said set further modifies the stability of said target molecule, wherein a further modification in stability is indicated by a further change in said unfolding curve.

4. The method of claim 3, wherein the screening of said one or more of a multiplicity of different molecules for their ability to modify the stability of the target molecule comprises:

(a) contacting said target molecule with one or more of said multiplicity of different molecules in each of a multiplicity of containers;

(b) treating said target molecule in each of said multiplicity of containers to cause said target molecule to unfold;

(c) measuring in each of said containers a physical change associated with the unfolding of said target molecule;

(d) generating an unfolding curve for said target molecule for each of said containers;

(e) comparing each of said unfolding curves in step (d) to (1) each of the other unfolding curves and/or to (2) the unfolding curve for said target molecule in the absence of any of said multiplicity of different molecules; and

(f) determining whether any of said multiplicity of different molecules modifies the stability of said target molecule, wherein a modification in stability is indicated by a change in said unfolding curve.

5. The method of claim 1, wherein said one or more co-regulators includes a co-activator and/or a co-repressor.

6. The method of claim 5, wherein one or more molecules of the set further modify the stability of the target molecule in the presence of a co-activator, thereby identifying an agonist of the target molecule when in the presence of the co-activator.

7. The method of claim 5, wherein one or more molecules of the set further modify the stability of the target molecule in the presence of a co-repressor, thereby identifying an antagonist of the target molecule when in the
5 presence of the co-repressor.

8. A method of identifying an agonist or an antagonist of a co-regulator-dependent target molecule comprising providing a set of molecules that shift the thermal unfolding curve of the target molecule and screening one or more of said molecules of said set for their ability to further shift the thermal unfolding curve of the target molecule in the presence of one or more co-regulators; wherein a further shift in the thermal unfolding curve of the target molecule in the presence of a molecule of said set and a co-regulator of said one or more co-regulators indicates whether the molecule is an agonist or an
10 antagonist of the target molecule when in the presence of said co-regulator.
15

9. The method of claim 8, wherein providing the set of molecules that shift the thermal unfolding curve of the target molecule comprises screening one or more of a multiplicity of different molecules for their ability to shift the
20 thermal unfolding curve of the target molecule.

10. The method of claim 9, wherein the screening of said one or molecules of said set of molecules for their ability to further shift the thermal unfolding curve of the target molecule comprises:

- 25 (a) contacting said target molecule and one or more molecules of said set with one or more of said co-regulators in each of a multiplicity of containers;
- (b) heating said multiplicity of containers;
- (c) measuring in each of said containers a physical change
30 associated with the thermal unfolding of said target molecule resulting from said heating;

- 36 -

(d) generating a thermal unfolding curve for said target molecule as a function of temperature for each of said containers;

5 (e) comparing each of said thermal unfolding curves in step (d) to (1) each of the other thermal unfolding curves and/or to (2) the thermal unfolding curve for said target molecule in the absence of (i) any of said molecules of said set and/or (ii) said co-regulators; and

(f) determining whether any of said molecules of said set further shift the thermal unfolding curve of said target molecule.

10 11. The method of claim 10, wherein the screening of said one or more of a multiplicity of different molecules for their ability to shift the thermal unfolding curve of the target molecule comprises:

(a) contacting said target molecule with one or more of said multiplicity of different molecules in each of a multiplicity of containers;

15 (b) heating said multiplicity of containers;

(c) measuring in each of said containers a physical change associated with the thermal unfolding of said target molecule resulting from said heating;

20 (d) generating a thermal unfolding curve for said target molecule as a function of temperature for each of said containers;

(e) comparing each of said thermal unfolding curves in step (d) to (1) each of the other thermal unfolding curves and/or to (2) the thermal unfolding curve for said target molecule in the absence of any of said multiplicity of different molecules; and

25 (f) determining whether any of said multiplicity of different molecules shift the thermal unfolding curve of said target molecule.

12. The method of claim 8, wherein said one or more co-regulators includes a co-activator and/or a co-repressor.

30 13. The method of claim 12, wherein one or more molecules of the set

further shift the thermal unfolding curve of the target molecule in the presence of a co-activator, thereby identifying an agonist of the target molecule when in the presence of the co-activator.

5 14. The method of claim 12, wherein the one or more molecules of the set further shift the thermal unfolding curve of the target molecule in the presence of a co-repressor, thereby identifying an antagonist of the target molecule when in the presence of the co-repressor.

10 15. A method of identifying an antagonist of a co-regulator-dependent target molecule comprising providing a set of molecules that modify the stability of the target molecule and screening one or more molecules of said set for their ability to further modify the stability of the target molecule in the presence of one or more co-activators; wherein no further modification of the stability of the target molecule in the presence of a molecule of said set and a co-activator of said one or more co-activators indicates that the molecule of said set is an antagonist of the target molecule when in the presence of said co-activator.

15 16. The method of claim 15, wherein providing the set of molecules that modify the stability of the target molecule comprises screening one or more of a multiplicity of different molecules for their ability to modify the stability of the target molecule.

20 17. The method of claim 16, wherein the screening of said one or molecules of said set of molecules for their ability to further modify the stability of the target molecule comprises:

25 (a) contacting said target molecule and one or more molecules of said set with one or more of said co-activators in each of a multiplicity of containers;

30 (b) treating said target molecule in each of said multiplicity of

containers to cause said target molecule to unfold;

(c) measuring in each of said containers a physical change associated with the unfolding of said target molecule;

5 (d) generating an unfolding curve for said target molecule for each of said containers;

(e) comparing each of said unfolding curves in step (d) to (1) each of the other unfolding curves and/or to (2) the unfolding curve for said target molecule in the absence of (i) any of said molecules of said set and/or (ii) said co-activators; and

10 (f) determining whether any of said molecules of said set further modifies the stability of said target molecule, wherein a further modification in stability is indicated by a further change in said unfolding curve.

18. The method of claim 17, wherein the screening of said one or more of
15 a multiplicity of different molecules for their ability to modify the stability of the target molecule comprises:

(a) contacting said target molecule with one or more of said multiplicity of different molecules in each of a multiplicity of containers;

20 (b) treating said target molecule in each of said multiplicity of containers to cause said target molecule to unfold;

(c) measuring in each of said containers a physical change associated with the unfolding of said target molecule;

(d) generating an unfolding curve for said target molecule for each of said containers;

25 (e) comparing each of said unfolding curves in step (d) to (1) each of the other unfolding curves and/or to (2) the unfolding curve for said target molecule in the absence of any of said multiplicity of different molecules; and

30 (f) determining whether any of said multiplicity of different molecules modifies the stability of said target molecule, wherein a modification in stability is indicated by a change in said unfolding curve.

19. A method of identifying an antagonist of a co-regulator-dependent target molecule comprising providing a set of molecules that shift the thermal unfolding curve of the target molecule and screening one or more of said molecules of said set for their ability to further shift the thermal unfolding curve of the target molecule in the presence of one or more co-activators; wherein no further shift in the thermal unfolding curve in the presence of a molecule of said set and a co-activator of said one or more co-activators indicates that the molecule of said set is an antagonist of the target molecule when in the presence of said co-activator.

5

10

20. The method of claim 19, wherein providing the set of molecules that shift the thermal unfolding curve of the target molecule comprises screening one or more of a multiplicity of different molecules for their ability to shift the thermal unfolding curve of the target molecule.

15

21. The method of claim 20, wherein the screening of said one or molecules of said set of molecules for their ability to further shift the thermal unfolding curve of the target molecule comprises:

20

(a) contacting said target molecule and one or more molecules of said set with one or more of said co-activators in each of a multiplicity of containers;

(b) heating said multiplicity of containers;

(c) measuring in each of said containers a physical change associated with the thermal unfolding of said target molecule resulting from said heating;

25

(d) generating a thermal unfolding curve for said target molecule as a function of temperature for each of said containers;

30

(e) comparing each of said thermal unfolding curves in step (d) to (1) each of the other thermal unfolding curves and/or to (2) the thermal unfolding curve for said target molecule in the absence of (i) any of said molecules of said set and/or (ii) said co-activators; and

- 40 -

(f) determining whether any of said molecules of the set further shift the thermal unfolding curve of said target molecule.

22. The method of claim 21, wherein the screening of said one or more of
5 a multiplicity of different molecules for their ability to shift the thermal unfolding curve of the target molecule comprises:

(a) contacting said target molecule with one or more of said multiplicity of different molecules in each of a multiplicity of containers;

(b) heating said multiplicity of containers;

10 (c) measuring in each of said containers a physical change associated with the thermal unfolding of said target molecule resulting from said heating;

(d) generating a thermal unfolding curve for said target molecule as a function of temperature for each of said containers;

15 (e) comparing each of said thermal unfolding curves in step (d) to (1) each of the other thermal unfolding curves and/or to (2) the thermal unfolding curve for said target molecule in the absence of any of said multiplicity of different molecules; and

(f) determining whether any of said multiplicity of different molecules shift the thermal unfolding curve of said target molecule.
20

23. A method of identifying an agonist of a co-regulator-dependent target molecule comprising providing a set of molecules that modify the stability of the target molecule and screening one or more molecules of said set for their ability to further modify the stability of the target molecule in the presence of one or more co-repressors; wherein no further modification of the stability of the target molecule in the presence of a molecule of said set and a co-repressor of said one or more co-repressors indicates that the molecule of said set is an agonist of the target molecule when in the presence of said co-repressor.
25

30 24. The method of claim 23, wherein providing the set of molecules that

modify the stability of the target molecule comprises screening one or more of a multiplicity of different molecules for their ability to modify the stability of the target molecule.

5 25. The method of claim 24, wherein the screening of said one or molecules of said set of molecules for their ability to further modify the stability of the target molecule comprises:

10 (a) contacting said target molecule and one or more molecules of said set with one or more of said co-repressors in each of a multiplicity of containers;

 (b) treating said target molecule in each of said multiplicity of containers to cause said target molecule to unfold;

15 (c) measuring in each of said containers a physical change associated with the unfolding of said target molecule;

 (d) generating an unfolding curve for said target molecule for each of said containers;

20 (e) comparing each of said unfolding curves in step (d) to (1) each of the other unfolding curves and/or to (2) the unfolding curve for said target molecule in the absence of (i) any of said molecules of said set and/or (ii) said co-repressors; and

 (f) determining whether any of said molecules of said set further modifies the stability of said target molecule, wherein a further modification in stability is indicated by a further change in said unfolding curve.

25 26. The method of claim 25, wherein the screening of said one or more of a multiplicity of different molecules for their ability to modify the stability of the target molecule comprises:

30 (a) contacting said target molecule with one or more of said multiplicity of different molecules in each of a multiplicity of containers;

 (b) treating said target molecule in each of said multiplicity of containers to cause said target molecule to unfold;

- 42 -

(c) measuring in each of said containers a physical change associated with the unfolding of said target molecule;

(d) generating an unfolding curve for said target molecule for each of said containers;

5 (e) comparing each of said unfolding curves in step (d) to (1) each of the other unfolding curves and/or to (2) the unfolding curve for said target molecule in the absence of any of said multiplicity of different molecules; and

10 (f) determining whether any of said multiplicity of different molecules modifies the stability of said target molecule, wherein a modification in stability is indicated by a change in said unfolding curve.

27. A method of identifying an agonist of a co-regulator-dependent target molecule comprising providing a set of molecules that shift the thermal unfolding curve of the target molecule and screening one or more of said molecules of said set for their ability to further shift the thermal unfolding curve of the target molecule in the presence of one or more co-repressors; wherein no further shift in the thermal unfolding curve in the presence of a molecule of said set and a co-repressor of said one or more co-repressors indicates that the molecule of said set is an agonist of the target molecule when in the presence of said co-repressor.

15

20

25

28. The method of claim 27, wherein providing the set of molecules that shift the thermal unfolding curve of the target molecule comprises screening one or more of a multiplicity of different molecules for their ability to shift the thermal unfolding curve of the target molecule.

29. The method of claim 28, wherein the screening of said one or molecules of said set of molecules for their ability to further shift the thermal unfolding curve of the target molecule comprises:

30

(a) contacting said target molecule and one or more molecules of said set with one or more of said co-repressors in each of a multiplicity of

containers;

- (b) heating said multiplicity of containers;
- (c) measuring in each of said containers a physical change associated with the thermal unfolding of said target molecule resulting from said heating;
- (d) generating a thermal unfolding curve for said target molecule as a function of temperature for each of said containers;
- (e) comparing each of said thermal unfolding curves in step (d) to (1) each of the other thermal unfolding curves and/or to (2) the thermal unfolding curve for said target molecule in the absence of (i) any of said molecules of said set and/or (ii) said co-repressors; and
- (f) determining whether any of said molecules of the set further shift the thermal unfolding curve of said target molecule.

15 30. The method of claim 29, wherein the screening of said one or more of a multiplicity of different molecules for their ability to shift the thermal unfolding curve of the target molecule comprises:

- (a) contacting said target molecule with one or more of said multiplicity of different molecules in each of a multiplicity of containers;
- (b) heating said multiplicity of containers;
- (c) measuring in each of said containers a physical change associated with the thermal unfolding of said target molecule resulting from said heating;
- (d) generating a thermal unfolding curve for said target molecule as a function of temperature for each of said containers;
- (e) comparing each of said thermal unfolding curves in step (d) to (1) each of the other thermal unfolding curves and/or to (2) the thermal unfolding curve for said target molecule in the absence of any of said multiplicity of different molecules; and
- (f) determining whether any of said multiplicity of different molecules shift the thermal unfolding curve of said target molecule.

31. A method for determining an agonist or an antagonist of a target molecule having an unknown function comprising:

providing a set of molecules that modify the stability of a target molecule having an unknown function, wherein said set of molecules modify the stability of receptors which share biological function, and screening one or more molecules of said set for their ability to further modify the stability of the target molecule in the presence of one or more co-regulators, wherein a further modification of the stability of the target molecule in the presence of a molecule of said set and a co-regulator of said one or more co-regulators indicates whether the molecule is an agonist or an antagonist of the target molecule when in the presence of said co-regulator.

32. The method of claim 31, wherein providing the set of molecules that modify the stability of the target molecule comprises screening one or more panels of molecules which modify the stability of receptors which share biological function for their ability to modify the stability of the target molecule.

33. A method for determining an agonist or an antagonist of a target molecule having an unknown function comprising:

providing a set of molecules that shift the thermal unfolding curve of a target molecule having an unknown function, wherein said set of molecules shift the thermal unfolding curve of receptors which share biological function, and screening one or more molecules of said set for their ability to further shift the thermal unfolding curve of the target molecule in the presence of one or more co-regulators, wherein a further shift in the thermal unfolding curve of the target molecule in the presence of a molecule of said set and a co-regulator of said one or more co-regulators indicates whether the molecule is an agonist or an antagonist of the target molecule when in the presence of said co-regulator.

- 45 -

34. The method of claim 33, wherein providing the set of molecules that shift the thermal unfolding curve of the target molecule comprises screening one or more panels of molecules which shift the thermal unfolding curve of receptors that share biological function for their ability to shift the thermal unfolding curve of the target molecule.

5

35. The method of any one of claims 1-34, wherein the target molecule is a nuclear receptor.

10

36. The method of any one of claims 1-34, wherein the target molecule is a G-protein coupled receptor.

37. The method of any one of claims 1-30, wherein the target molecule is ER- α .

15

38. The method of any one of claims 1-30, wherein the target molecule is ER- β .

20

39. The method of any one of claims 1-30, wherein the target molecule is a tyrosine kinase.

40. The method of any one of claims 1-30, wherein the target molecule is a NF- κ B.